REMARKS

I. Status of the Claims

Claims 1, 11, 19, 22 and 24-27 are pending in the application. Claims 11 and 26 stand withdrawn from consideration as drawn to a non-elected invention. Claims 1, 19 and 22 stand rejected, and claims 24, 25 and 27 stand newly rejected under 35 U.S.C. §112, first paragraph as lacking written description, and claims 1, 19 and 22 stand rejected, and claims 24, 25 and 27 stand newly rejected, also under §112, first paragraph, as lacking enablement. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

II. Enablement

Claims 1, 19, 22, 24, 25 and 27 remain rejected as allegedly lacking enablement, for reasons of record. The examiner provides a point by point rebuttal of applicants' previous arguments. However, each of the counter-arguments advanced by the examiner are flawed, as explained below.

A. Evidence of Intracellular Expression Is Relevant to Enablement

Applicants had urged that the examiner had improperly dismissed evidence of record regarding the expression of ferritin-H using genetic transformation. Moreover, evidence was pointed out showing (a) the ability of ferritin-H to selectively bind to the β-globin promoter, as contrasted to other ferritins, demonstrated in *in vitro* assays; (b) that even a proteolytically generated fragment of ferritin-H can bind effectively to the β-globin promoter *in vitro*; and (c) that transient expression of a GFP-labeled ferritin-H localizes in the nucleoplasm of cells.

In response, the examiner argues that "protein therapy and nucleic acid gene therapy are two distinct fields of scientific study, and such is reflected in the patent classification system." While possibly true, the *in vitro* ability of the ***protein*** to provide the relevant biological effect eliminates numerous variables that allegedly impinge upon enablement of the present claims. This much is shown.

Next, the examiner sets out a series of questions that allegedly must be answered to establish enablement. "[T]he standard for evaluation is whether the ferritin H could be efficiently delivered (exposed) to the nucleus of the globin-producing cells in a significant amount into a significant population of cells, and whether such delivery would lead to suppressing sickle cell disease and correction of the disease phenotype." Simply put, the examiner is wrong.

First, the standard is whether one of ordinary skill in the art, reading the specification, would find that the specification adequately teaches how to make and use the claimed invention. Second, if ferritin H can be delivered to the nucleus of sufficient cells, the evidence of record ***already show the necessary biological effect***. Thus, the question posed by the examiner remains one ***only*** of delivery. And third, there is ***no*** requirement for ***correction*** of the disease phenotype – ***treatment*** need not provide a ***cure***. The remaining comments about pharmacologic behavior and long term "consequences" are ***not*** issues within the purview of the PTO, much less are they necessary to address enablement. Rather, they are the domain of the FDA, and such regulatory concerns should be left to that agency. *See In re Krimmel*, 130 USPQ 215, 219 (CCPA 1961).

Next, it is argued that delivery of a heteromeric ferritin shell of 24 subunits is, at a minimum, a challenging endeavor for which enablement is to be questioned. However, one of ordinary skill in the art would hardly leap to the conclusion that this is what is proposed. For

example, page 35 of the specification states "Delivering the ferritin-H peptide or a truncated form of it to erythroid precursor cells is a more effective, more natural form of therapy than the partial measures currently in use to treat sickle cell disease and β-thalassemias. Similar delivery of ferritin-derived peptides provides effective treatments and protection in Alzheimer's and other neurodegenerative disease and cancers." Thus, it is clearly stated that a peptide that is only part of a single subunit could be delivered, and thus the inventors also clearly contemplated delivery of subunits and not necessarily a heteromeric complex. It is likely that a single subunit (21 kD) or peptide fragment thereof (12-14 kD) will not require a special transport system, and will avoid the endosomal system. Once in the cytoplasm, Thompson *et al.* (abstract, International BioIron Congress, Sorrento, Italy, 1999; attached) teach that "H ferritin was selectively taken up into the nucleus whereas extracellular L ferritin remained cytoplasmic."

The examiner admits that "Of course, it is entirely possible to deliver a macromolecule protein across the plasma membrane and into the nucleus of a cell ...." and states that "Schwarze *et al.* (*Trends Cell Biol*, 2000 July; 10:290) teach three protein-transduction domains (PTDs) that could be linked to a desired peptide for delivery to all tissues." However, she goes on to argue that "Currently, there are only a handful of papers in the literature that describe the successful transduction of full-length proteins and report a phenotype." This statement is entirely unsupported on the record. Further, the examiner states "Since the art of record (*e.g.*, Harrison *et al.*) teaches that Ferritin is ubiquitously distributed in many different types of cells and living species, not limited to the globin-producing cells, it is important to deliver it selectively." But Harrison *et al.* teaches that ferritin H is ***not equally*** distributed and is highly expressed in only a few cells and tissues, *e.g.*, embryos, adult heart, brain and kidney, thus undercutting the examiner's position further.

It also is argued that "... the specification fails to teach any ligand specific for globin-producing cells." However, this is well known in the art. Possible ligands to add to ferritin H to target it to red cells include (1) an erythropoietin fragment, (2) a transferrin fragment, or even (3) the *tat* protein. These concepts are just a few of the ways that ferritin H delivery can be achieved. It is likely that any number of ligands could be found that would not alter the desired DNA-binding function of ferritin H. Indeed, the inventors have found (recent results) that a GFP-FtH fusion protein tracks to the nucleus, showing that even adding such a large tag as GFP does not disturb nuclear localization in CV-1 cells.

Thus, the examiner's argument that "The specification fails to address any one of the questions as discussed by the skilled artisans" is simply incorrect. As discussed, the "questions" posed by the Examiner may not be problems at all, and none would be insurmountable obstacles for anyone wishing to apply the technology.

The examiner states that, in addition, "host immune response would be another concern for ferritin-H therapy because the nature of the sickle cell disease determines that such protein therapy is a long-term one." Once again, the examiner is incorrect. A host immune response to ferritin-H is ***highly*** unlikely even in the long term. The reason for this, as taught in the art (Harrison *et al.*), is that there ferritin is circulating in the blood of all humans for their entire life span. Although this circulating ferritin is composed almost entirely of ferritin-L chains, it is clear that the immune system has been educated since embryonic stages to accept the presence of both ferritin-H and ferritin-L as "self." Therefore, this too is a fallacious argument. Rather, this is one of the ***advantages*** to the use of ferritin-H protein as a therapeutic agent. It is unlikely that ferritin-H will cause any immune response in the patient.

The examiner next raises the question of effects of ferritin-H for the long-term in sickle cell disease. Since the proposed method of use of ferritin-H is to affect globin gene expression during the early stages of erythroid differentiation when globin gene expression is occurring, there is no need for ferritin H to be present constantly. In reality, ferritin-H is needed for only a very short time compared to the 120-day life span of a red blood cell that would no longer sickle. Thus, application of the ferritin-H protein would be infrequent, possibly only three or four times per year. Furthermore, as the examiner acknowledges (above), the half-life of proteins is short and turnover is frequent. So, any side effects would not persist, while the ***beneficial*** effect of ferritin-H on preventing sickling would persist for 120 days since there is no hemoglobin turnover inside the red cell during that time. Thus, the examiner's concerns are not reasonable when viewed in light of the state of knowledge that existed at the time of the patent application.

Next, the examiner states "Thus, it is important to look at the nature of the sickle cell diseases, the underlying mechanism of pathogenesis, the treatment strategy, and the complexity of the target that ferritin-H affects." It should be clear to one skilled in the relevant art that most of this statement is irrelevant to the method proposed for treatment. The complex pathogenesis of sickle cell disease is a cascade of events and morbidity resulting from the properties of the diseased red cell which in turn are the result of the concentration of mutated beta-globin in the cell. Prior art referenced in the patent application has demonstrated ***proof of principle*** for the concept that decreasing the concentration of the mutated beta-globin in the differentiating red cell will result in a relative increase in gamma-globin in its place. The result is a decrease of mutated (sickle) hemoglobin to the point that sickling can no longer occur, and the downstream events that represent the complicated pathology of sickle cell disease also no longer occur. Thus, the target for the ferritin-H effects is a transient one, present for only a short time at the

beginning of the genesis of the adult red cell. Ferritin-H protein will not last long beyond its needed time – as globin gene expression increases, adult erythroid precursor cells degrade all their ferritin (Vaisman *et al.*, 2000; *Br J Haematol* 110:394-40). Thus, concerns about downstream events are not relevant.

The examiner states that "Currently, the treatment strategy is to supply natural antisickling hemoglobins such as fetal hemoglobin (gamma globin) and normal beta-globin to correct the diseased polymerization and prevent sickle cell formation." That is exactly what the inventors are proposing to do. They have shown proof that ferritin-H will act as a repressor of the diseased gene (the beta-globin gene), thereby decreasing its concentration in the red cell through a known mechanism of gene expression. Previous work suggests that there will be a compensatory increase in fetal hemoglobin (gamma globin) when beta-globin decreases. Thus, implementation of the proposed invention would not be questioned by those skilled in the art.

The examiner next states that "Applicants now proposed a new treatment strategy, *i.e.*, suppress the production of the mutated beta-globin using the gene repressor ferritin-H. This strategy requires an excessive amount of ferritin-H ...." The examiner then goes on to discuss literature cited in the application, *i.e.*, Picard *et al.*, citing only the abstract of that paper, and noting reduced accumulation of beta-globin that results from over-expression of ferritin-H. The examiner then questions the consequences of the overall impaired hemoglobin synthesis reported. Reading beyond the abstract of this paper, the question is answered. Although Picard *et al.* did not quantify their data, they assume that most of the "impaired hemoglobin synthesis" was due to decreased beta-globin. It is impossible to know from Picard *et al.* whether adequate overall hemoglobin production would be achieved since the erythroleukemia cells they were using never become fully hemoglobinized, as compared to normal human adult red cells. Thus,

little weight should be placed on concerns based on this paper. The examiner also wonders about the effect of the decrease in ferritin L with overexpression of ferritin H. But Picard *et al.* show that the main effect of overexpression of ferritin H on cellular iron status is to ***lower the labile iron pool (LIP).*** This is also a good effect in terms of sickle cell disease, in which build-up of free iron causes much damage; over-expression of ferritin-H ***lowers*** free iron.

Next, the examiner asks, "More importantly, what is the consequence of long-term suppression of beta-globin? The specification is silent this aspect." Not so. Once again, prior studies referenced in the application address the long-term supression of beta-globin – showing long-term increased gamma-globin and a life free of the symptoms and damaging effects of sickle cell disease. Perrine *et al.*, 1978; *Ann Intern Med* **88:**1-6.

The examiner states "However, it is noteworthy there is a type of beta-chain hemoglobinopathy named beta-thalassemia, which arises from the total or partial reduction in synthesis of structurally normal beta-globin chain. Lack of beta-globin synthesis results in precipitation of free alpha-globin chains and the subsequent destruction of erythroid precursors in the bone marrow and the spleen (see *e.g.*, *Herzog et al,* Expert Rev Cardiovascular Ther 2003; 1:215-32). Thus, theoretically, long-term administration of exogenous ferritin-H may lead to another disease, beta thalassemia." As will be discussed below, this is not correct.

First, thalassemia is ***not*** a hemoglobinopathy. As defined in hematology textbooks available before and after the application, *hemoglobinopathies* refers to a set of disorders resulting from mutations within the coding sequences of globin genes, whereas *thalassemias* refers to a class of diseases resulting from decreased synthesis from otherwise normal globin genes. Thus, the two classes of diseases are distinct in both terminology and origin, and for that reason, chapters and treatises discussing hemoglobin diseases usually have titles with both terms

(hemoglobinopathies and the thalassemias) in their titles. But when and wherever the term hemoglobinopathies is used in the application/specification, it refers to hemoglobinopathies and ***not*** to thalassemias.

Second, it is highly unlikely that application of ferritin-H to accomplish a phenotypic cure for sickle cell disease will result in a beta-thalassemia phenotype, for several reasons. In most humans, including most patients with sickle cell disease, a repression of beta-globin leads to at least a partial compensatory increase in gamma-globin, resulting in at most a mild excess of alpha chains and a phenotype that is asymptomatic, *i.e.*, a very mild "thalassemia" of no clinical consequence and requiring no treatment. See Dover & Boyer, 1987, *Blood* 69:1109-13; Rodgers *et al.*, 1989, *Prog Clin Biol Res* 316B: 281-93. Ferritin-H, in addition to being a proven repressor of beta-globin production, has been reported to be a stimulator of gamma-globin expression. Wu & Noguchi, 1991, *J Biol Chem* 266:17566-72. Therefore, the decrease in mutated beta-globin expression will likely be replaced completely with gamma-globin production, due to the dual nature of gene regulation by ferritin-H. The phenotypic cure provided by short-term ferritin-H treatment does not require full repression of beta-globin production - in fact, far less than full repression is required. It is known from prior work that a beta-to-gamma switch of 25-30% is sufficient to reduce the probability of sickling of the red cell to near zero, *i.e.*, to be sufficient to affect a phenotypic cure. Noguchi *et al.*, 1988, *N Eng J Med* 318:96-99; Poillon *et al.*, 1993, 90 (11):5039-43. So, even if the amount of gamma (fetal)-globin production does not ***exactly*** match or fully compensate for the amount of beta-globin suppression, the chain imbalance is very likely to be a very small percent of the total and clinically insignificant, *i.e.*, the phenotypic cure of sickle cell will still be achieved without significant side-effects.

The examiner quotes the Federal Circuit stating "[A] specification need not disclose what is well known in the art .... It is the specification ... that must supply ***the novel aspects of an invention*** in order to constitute adequate enablement" (emphasis added). As discussed in the specification, the novel aspect of the invention is the application of ferritin-H to affect a partial adult-to-fetal hemoglobin switch in the differentiating red blood cells of sickle cell patients. It is well known in the art that this can be accomplished, and there is more than sufficient information on the procedures and materials by which that can be accomplished.

The examiner also notes that "... applicants mentioned data from the inventors' laboratory. However, such data have not been properly identified, thus the Office could not evaluate the additional data. Applicants now attach the declaration of Robert Broyles, one of the inventors. Therein, data are presented showing induction of the endogenous FtH gene in human cells (NT-2 cells; K562 cells) by abscissic acid. Further, initial data from a transgenic mouse made to express human FtH shows that expression of the mouse beta-major globin gene is repressed relative to the beta-minor gene, which cannot be repressed because it lacks the FtH repression site).

The examiner also suggests submission of *in vitro* or *in vivo* evidence showing that administration of exogenous ferritin-H protein to the nucleus of a cell will achieve a therapeutic effect, but then admits that "...this alone would not fail the test of enablement." The examiner is quite right. Experimental data, much less those showing therapeutic effects, are not required under the law.

The examiner states that:

> The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which that claimed invention pertains, then there is predictability in the art. On the other hand, if

> one skilled in the art cannot readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is a lack of predictability in the art. Accordingly, what is known in the art provides evidence as to the question of predictability.

Page 13. It is applicants' position that the information provided in the specification, along with citations to related art (*e.g.*, Wu & Noguchi, *JBC* **266:** 17566, 1991), clearly provide the requisite predictability, *i.e.*, those skilled in the art, once they realize that ferritin H will act as a gene regulator and change the ratio of fetal to adult hemoglobin in differentiating sickle cells, can readily exploit the effect of this gene regulation to treat sickle cell anemia. This is ***predictable*** because such an effect occurs in people who have sickle cell plus HPFH (hereditary persistence of fetal hemoglobin) – an naturally-occurring version of the proposed treatment. Given this example, those skilled in the art would readily accept that one can move from the present application to a treatment.

The examiner apparently remains concerned about delivery of ferritin H to the nucleus, even when ferritin H is bathing cells in an *ex vivo* embodiment. Given that it is likely that the 21 kD protein - the monomer chain that constitutes ferritin-H, or an even smaller peptide derived therefrom - will transverse the cell membrane without a specialized carrier, then it is only a matter of transport to the nucleus from the cytoplasm that remains. Thompson *et al.* teach that, once in the cytoplasm "H ferritin was selectively taken up into the nucleus whereas extracellular L ferritin remained cytoplasmic" (abstract, International BioIron Congress, Sorrento, Italy, 1999, attached). As stated by the examiner, "each protein has its distinct physical and biological property" It is the uniqueness of each one that makes its use and its target very specific, as in this case. This uniqueness of each protein drug insures that there will be few if any side effects. Because of their specificity, only proteins (and the genes that encode them), of all the biological molecules, have this potential for targeted effect and few side effects.

In response to examiner's concern that "the half-life of a protein is short...," applicants submit that one will keep adding ferritin-H to the solution bathing the cells until sufficient changes in globin gene expression have occurred, then one will let it "die." This is an advantage for *ex vivo* embodiments since the ferritin will be gone when the differentiated, expanded population of red cells is re-infused into the patient; there will be no side-effects on non-erythroid cells since they will not be exposed to the ferritin, but the red cells whose phenotype was changed as they differentiated *ex vivo* will remain in the non-sickling state for approximately 120 days. This is the type of extrapolation that one skilled in the art would naturally make or already know.

In rebuttal to applicants' challenge of the previous actions, the examiner stated again that "... hurdles in protein therapy and protein nucleus delivery as taught by the skilled artisan, *e.g.*, Buckel," along with "the disconnection between basic molecular research and clinical therapy such as taught by Mankad *et al.*" demonstrate too much unpredictability. However, it is disputed that Mankad provides support for this statement. Rather, Mankad has simply noted that the three most effective treatments for sickle cell are (1) prophylactic penicillin which has decreased the incidence of acute chest syndrome (due to peumococcal infections, as a major side effect of the main problem of sickle cell disease), (2) transfusions (replace sickled blood periodically) and other supportive measures, and (3) hydroxyurea therapy which has decreased acute painful episodes, acute chest syndrome, and the need for blood transfusions in adults. The latter was intended to promote reverse hemoglobin switching and increase fetal hemoglobin, but all of its effects and the molecular mechanisms behind them are still poorly understood. Furthermore, Mankad states (in the abstract) that "The benefits and risks of hydroxyurea for younger children and long-term risks in all patients will be evaluated in future investigations."

Thus, Mankad makes the point that much is yet to be done towards effective treatments or cures for sickle cell disease, especially in applying all the molecular knowledge we have acquired. His statements were ***not*** intended to say that outcomes of applying molecular regulation are unpredictable. Rather, it is clear that key information is missing at the time of his writing and its publication. It is worth noting that the date of publication of Mankad's review was the Jan./Feb., 2001, issue of Pediatric Pathology and Molecular Medicine, 6 months ***before*** the appearance of the PNAS paper upon which this application is based (dated 31 July 2001).

Moreover, applicants challenge the general statement about the "unpredictability of chemical reactions," as ferritin H has only been shown to do two things, (1) chelate and oxidize iron and (2) bind DNA (and thereby affect gene expression). These actions are highly ***predictable.***

In response to applicants' argument that the only remaining question is one of protein delivery, the examiner discusses the work of Meyron-Holtz *et al.*, regarding the uptake of extracellular ferritin by human erythroid precursors. However, the paper and its conclusions are over-interpreted. In ***neither*** of their papers did Meyron-Holtz *et al.* ***show*** that ferritin taken up was "degraded by proteolysis in a lysosomal-like acidic cellular compartment." Rather, as stated in the introduction of *Blood* **94:**3205-11, 1999 (attached) they ***presumed*** that the "mechanism of ferritin iron mobilization is protein degradation by acid proteases in an acidic compartment in the cell." While the rather general protease inhibitor chloroquine and the cysteine protease inhibitor leupeptin inhibit the ferritin degradation, it was by no means proven that these drugs acted via the lysosomal compartment. The degradation takes a long time and is measured over days (0 to 48 hours), and as such, degradation by another pathway such as the proteosome could be involved as the normal pathway. Because they did not use proteosome inhibitors in their studies,

the answer remains unclear. Since ferritin has a short half-life in these cells (see references quoted by Meyron-Holtz *et al.*), they could be looking at another degradation pathway other than the lysosomal one.

What ***is*** clear is that apoferritin taken up by the cells lasts long enough to chelate iron and lower the labile iron pool, something it could not do from inside a lysosome. So, it is likely that ferritin taken up by these cells (especially apoferritin) ***does*** spend some time in the cytoplasm and, therefore, quite possibly, the nucleus. Once cannot know for sure because these workers did not look at these compartments; rather, they ***presumed*** that their interpretation was correct, when in fact they do not know. However, Thompson *et al.* teach that ferritin H in the cytoplasm will be taken into the nucleus. Apo-ferritin-H is the form that would be chosen first to apply to these cells to affect a change in gene expression. The inventors and others skilled in the art could also use protease inhibitors, as necessary, to prolong the time ferritin H would have to act. These things are easily done and do not constitute undue experimentation.

The examiner further states, on page 18, that "the criteria for enablement is the claimed subject matter has been fully enabled 'at the time the application was filed .... The fact that the specific protocol is not disclosed in the specification does not support the claims as filed." The examiner grossly misinterprets the enablement requirement by asking for a specific, detailed clinical protocol. Such is not needed to support a patent application under §112, first paragraph. To the contrary, applicants have provided the necessary information on ferritin H's biological effects, including the site of action, and the specification and prior art provide the remaining information

The examiner also argues that the now known fact of nuclear transport is missing from the specification as filed. This is ***irrelevant*** to enablement, since nothing additional is required in

order to perform the invention. In other words, no ***step*** or ***element*** has been omitted. Rather, the information on nuclear transport merely indicates that the specification as filed is indeed enabling, as the inventors predicted.

The examiner argues that "Hundreds and thousands of *in vitro* and *in vivo* studies have been performed before insulin and GM-CSF being used in clinical treatment." No information to support this allegation is provided. More relevant, however, is the question of whether these "hundreds and thousands" of studies were performed ***before*** patents covering the use of insulin or GM-CSF were granted? In summary, the examiner seeks to require positive proof of the methods now being claimed. However, this has never been the standard for patentability under §112, first paragraph. Rather, the reasonable predictability advanced by applicants here is more than sufficient reason to find the enablement requirement has been satisfied.

III. Anticipation

Claims 1, 19 and 27 are rejected under 35 U.S.C. §102(b) as anticipated by Adams *et al.*, as evidenced by Atkinson *et al.* and Sowemimo-Coker. The examiner indicates that the record, upon review, contains insufficient evidence to support applicants' prior statements that adult blood does not contain ferritin-H. Applicants traverse.

The examiner reasons that, since Atkinson *et al.* "teach" that ferritin is present in red blood cells, and since Sowemimo-Coker "teaches" that lysis of RBCs occurs during processing, then Adams *et al.* anticipate instant claims when they "teach" preventing a first stroke of sickle cell anemia in children by red blood [cell] transfusion – allegedly because they are giving ferritin to the patient via lysed RBCs. This is incorrect.

First, Atkinson *et al.* is misquoted twice. The method of isolating ferritin from blood cells in this paper uses (a) cells from a cold-blooded, lower vertebrate, not a human, and (b) the blood cells from which the ferritin was isolated were ***embryonic*** red blood cells of a bullfrog tadpole (a larva), ***not*** adult red blood cells. Thus, this paper in no way supports the rejection.

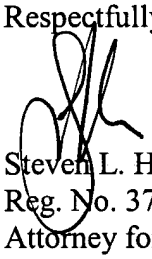
Second, the examiner has apparently ignored the fact that adult human circulating red blood cells contain no ferritin, even though the prior art demonstrating this for ***all vertebrates examined including mammals*** was cited and discussed in the instant specification. Prior publications clearly show that circulating adult human red blood cells contain ***no ferritin***; these cells lose the little ferritin that they had at the beginning of their differentiation, well before they leave the bone marrow and enter the circulation. *See* Theil, 1987; *Ann Rev Biochem* 56:289-315; Dickey *et al.*, 1987; *J Biol Chem* 262:7901-07.

Therefore, examiner's deduction that Adams *et al.* were delivering ferritin via a RBC transfusion is clearly wrong. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

IV. Conclusion

In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Should the examiner have any questions regarding this response, a telephone call to the undersigned is invited.

Respectfully submitted,



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